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From: Qazi, Sabiha
Sent: Saturday, June 01, 2002 2:10 PM
To: STIC-Biotech/ChemLib
Subject: Request for the references

Please provide following references.

1. Nestler JE, Barlascini CO, Clore JN, Blackard WG, Dehydroepiandrosterone Reduces Serum Low Density Lipoprotein Levels and Body Fat but does not Alter Insulin Sensitivity in Normal Men, *J. Clin Endocrinol Metab*, 66: 57-61, 1988.
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Thank you.

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Communication

Inhibition of Spontaneous Breast Cancer Formation in Female C3H (A^{vv/a}) Mice by Long-Term Treatment with Dehydroepiandrosterone¹

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ABSTRACT

Long-term p.o. treatment with dehydroepiandrosterone, an adrenal steroid found in subnormal plasma concentrations in women predisposed to develop breast cancer, inhibits the formation of spontaneous mammary cancer in female C3H (A^{vv/a}) mice.

INTRODUCTION

On the basis of a 10-year prospective study involving over 5000 apparently healthy women, Bulbrook *et al.* (3) concluded that women with subnormal excretory rates of androsterone and etiocholanolone experience an increased risk of breast cancer. Urinary androsterone and etiocholanolone are derived primarily from the adrenal steroid DHEA,² and subnormal excretory rates of these 2 steroids reflect low plasma concentrations of DHEA (26).

We have reported that DHEA protects cultured rodent cells against DMBA- and aflatoxin B₁-induced cytotoxicity and transformation and inhibits the metabolism of [³H]DMBA to water-soluble products (24). It is well established that DHEA is a potent noncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (16, 18, 22), the enzyme responsible for generating the bulk of extramitochondrial NADPH (9). Chemical carcinogens such as DMBA and aflatoxin B₁ require metabolic activation by NADPH-requiring mixed-function oxidases (17), and we have postulated that DHEA protects cultured cells against the toxic and transforming effects of these carcinogens by inhibiting their activation as a result of a lowering of NADPH levels.

Recently, Yen *et al.* (29) found that long-term DHEA treatment of VY (A^{vv/a}) mice significantly reduced their rate of weight gain without affecting their rate of food consumption, presumably by inhibiting lipogenesis as a result of inhibition of glucose-6-phosphate dehydrogenase.

We report now that long-term DHEA treatment of female C3H (A^{vv/a}) mice, in addition to reducing their weight gain, inhibits the formation of spontaneous breast cancer.

MATERIALS AND METHODS

Breeding pairs of C3H (A^{vv/a}) FB mice were obtained from

The Jackson Laboratory, Bar Harbor, Maine. These mice were mated to produce a large supply of males, which were then crossed with female C3H (a/a) mice (Skin and Cancer Hospital, Temple University, Philadelphia, Pa.), which carry the mammary tumor virus. The viable yellow females, C3H (A^{vv/a}), produced from this cross also contain the mammary tumor virus. As groups of 8 weanling C3H (A^{vv/a}) females (born within the same week) became available, they were divided into 2 groups and housed in plastic cages with 4 animals/cage. One group received 450 mg of DHEA per kg (prepared as a suspension in sesame oil)³ by p.o. intubation 3 times weekly, and the other received the sesame oil vehicle. The DHEA was obtained from Sigma Chemical Co., St. Louis, Mo. A total of 26 control and 24 DHEA-treated animals were used. Purina laboratory chow (11% fat) and water were provided *ad libitum*. The animal quarters were maintained at 24 ± 1° and at 40 to 45% relative humidity with 12 hr of light and 12 hr of darkness each day. All mice were identified by earmark and were weighed and palpated for breast cancer weekly. Fresh food was provided each week, and the food consumption of each group was determined by subtracting the residue at the end of one week from the amount of food initially added.

Breast Tumors. Mammary tumors were removed from moribund animals at autopsy, fixed in 10% buffered formalin, and stained with hematoxylin and eosin for histological examination.

Histochemical Measurement of Mammary Gland Glucose-6-phosphate Dehydrogenase. In order to determine if p.o. DHEA treatment inhibits mammary gland glucose-6-phosphate dehydrogenase, 3 female C3H mice received 450 mg of DHEA per kg by p.o. intubation, and 3 mice received sesame oil. Three, 6, and 24 hr later, groups of 2 mice were killed, and the glucose-6-phosphate dehydrogenase activity of mammary tissue was measured histochemically (14).

Statistical Analysis. Differences in mammary tumor incidence were evaluated by the χ^2 test with Yates' correction; differences in body weights, by Student's *t* test; and differences in the cumulative food consumptions, by the paired *t* test.

RESULTS

As illustrated in Chart 1, C3H (A^{vv/a}) mice receiving DHEA 3 times weekly gain weight at a rate significantly reduced from that of sesame oil-treated controls ($p < 0.001$ at all points after 9 weeks of age) even though the cumulative

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² The abbreviations used are: DHEA, dehydroepiandrosterone; DMBA, 7,12-dimethylbenz(a)anthracene.

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food consumptions of the 2 groups are not significantly different (Chart 2; $p = 0.134$).

Substantial inhibition of mammary gland glucose-6-phosphate dehydrogenase activity was observed at 3, 6, and 24 hr after p.o. DHEA treatment (Fig. 1, 24 hr). According to Yen *et al.* (29), DHEA-treated mice experience significantly reduced lipogenesis rates, and their reduced body weight is largely due to decreased deposition of triacylglycerol.

The cumulative breast cancer incidence in the control mice as of 9 months of age is shown in Chart 3. As of this time, no tumors have yet appeared in the DHEA-treated animals ($p < 0.001$).

DISCUSSION

It is well documented that laboratory mice and rats maintained on a calorically restricted diet experience a tumor incidence lower than that of those fed *ad libitum* (1, 25). The food-restricted rodents, which weigh less than their unrestricted counterparts largely as a result of the absence of excess body fat, also have an extended life span and a delayed onset of major degenerative diseases, including neoplasms (1).

A metabolic pattern in mice leading to increased fat deposition and a lowered food requirement per unit of weight gain results in an increased susceptibility to a variety of different spontaneous tumors. The yellow (A^y) and viable yellow (A^{vy}) mutations of the agouti locus, which produce such a metabolic pattern (7), confer an enhanced susceptibility to spontaneous breast cancer (11), hepatoma (11), leukemia (6), and lung adenoma (10). In humans, obesity is also positively correlated with the presence of cancer of the corpus uteri (8) and of the breast (12).

Long-term DHEA treatment also produces a very significant reduction in the rate of weight gain of male and female C3H (a/a) mice without affecting their rate of food consumption (unpublished observations). Preliminary data indicate that the DHEA-treated female C3H (a/a) mice experience a decreased incidence and rate of appearance of

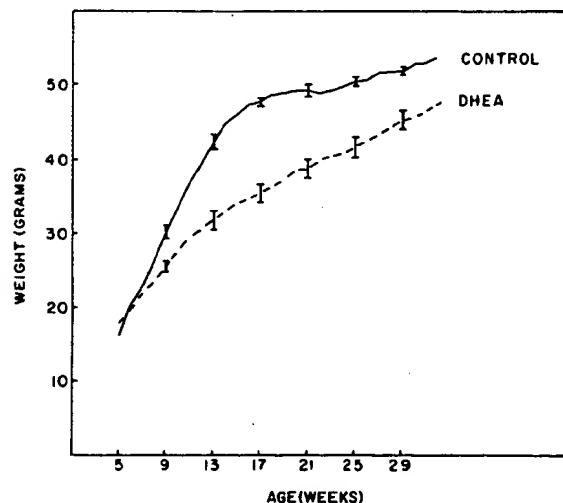


Chart 1. Growth curves for control (26) and DHEA-treated (24) mice. Points, weekly determinations of mean weights with the S.E. indicated at monthly intervals.

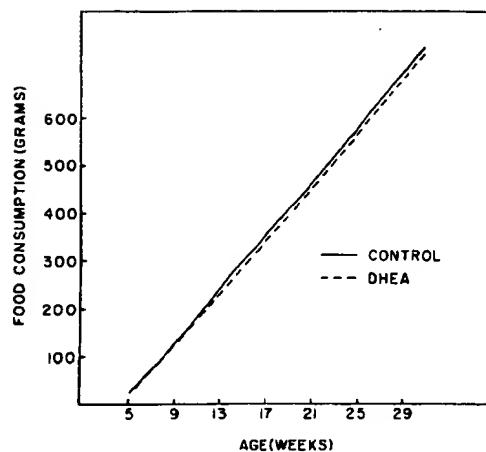


Chart 2. Cumulative food consumptions for control and DHEA-treated mice. Weekly food consumptions were computed by determining the total food consumption for each group of mice and dividing by the number of mice in each group.

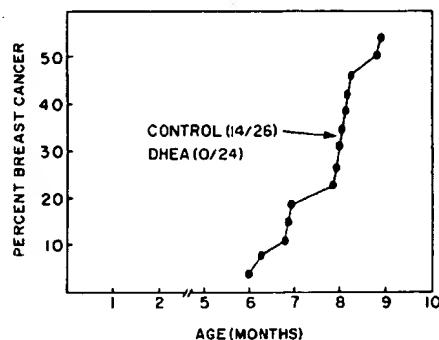


Chart 3. Cumulative breast cancer incidence over a 9-month period. Each breast tumor was recorded at the time of first palpation.

breast cancer. The tumors that have appeared thus far seem to grow equally well in the control and DHEA-treated mice, suggesting that hormone treatment has little effect on tumor growth. Potent androgens such as testosterone propionate also appear to have no apparent effect on the growth of spontaneous breast tumors in C3H mice (13, 21).

In addition, by 14 months of age the hair coats of the DHEA-treated C3H (a/a) mice show less coarsening and greying than the controls, and the steroid-treated mice appear younger looking. Inhibition of weight gain by food restriction is well known to delay the appearance of age-associated pathology and to extend life span.

DHEA-sulfate is the most abundant steroid hormone circulating in the plasma of humans, yet its biological function is obscure (26). After the second decade of life, the plasma concentration of this steroid declines, reaching levels in the very old of about 5% of its maximum value (19).

Several retrospective studies have reported subnormal plasma levels of DHEA-sulfate (2, 23, 28) and subnormal excretion rates of androsterone and etiocholanolone (5, 15) in women with advanced breast cancer. In patients with early breast cancer, however, results are more equivocal (4, 20, 27). Brownsey *et al.* (2), although detecting subnormal plasma concentrations of DHEA-sulfate in advanced breast cancer patients, found no abnormality in women with early disease. Wang *et al.* (28) measured plasma DHEA-sulfate

levels in women with early breast cancer on the day before mastectomy and again 10 to 14 days after surgery. They found no significant differences in plasma DHEA-sulfate concentrations between the controls and breast cancer patients in the preoperative samples, but the mean postoperative DHEA-sulfate level was clearly subnormal. The authors concluded that preoperative stress might have accounted for the elevated DHEA-sulfate levels in the preoperative patients. Rose *et al.* (23), on the contrary, found unequivocal subnormal mean plasma levels of DHEA-sulfate in both preoperative and postoperative early breast cancer patients as well as in those with advanced disease. Bulbrook *et al.* (3), on the basis of a large-scale prospective study, concluded that these hormone abnormalities precede the onset of breast cancer and may exist up to 9 years before diagnosis.

It is possible that the inhibition of breast cancer formation by DHEA as reported here may be related to the above clinical findings.

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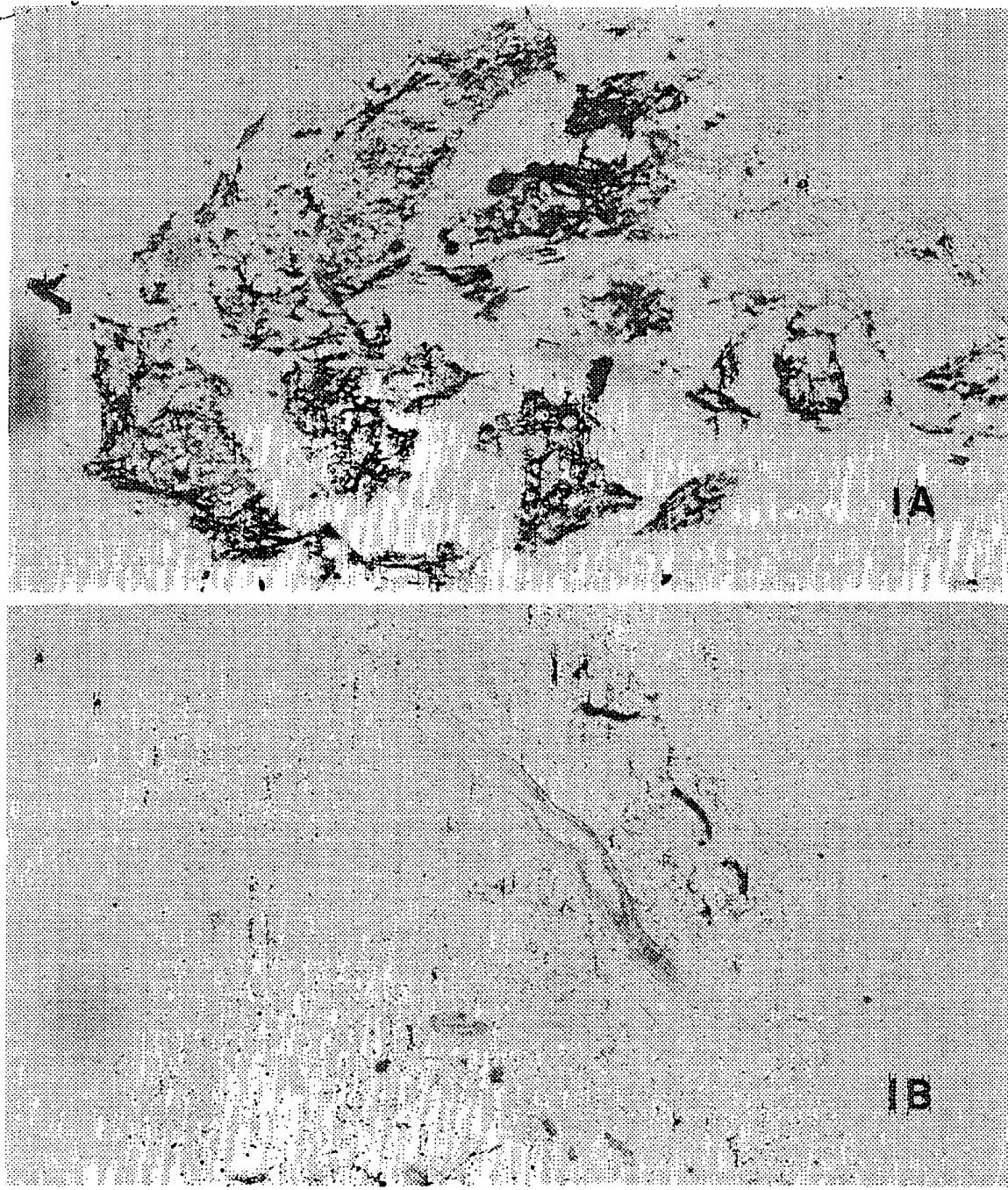


Fig. 1. Histochemical measurement of mammary gland glucose-6-phosphate dehydrogenase activity 24 hr after p.o. treatment with sesame oil vehicle (A) and with DHEA (450 mg/kg) suspended in sesame oil (B). $\times 40$.